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Genome instability: when RNA meets chromatin

Instabilité du génome: lorsque l'ARN rencontre la chromatine

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Rapport sur la Conférence
Conference Report

French summary/Résumé en français

La conférence Jacques Monod "**Instabilité du génome : Lorsque l'ARN rencontre la chromatine**", s'est tenue à Roscoff du 16 au 20 septembre 2019. Cette conférence organisée par Andres Aguilera (Président) et Gaëlle Legube (Vice-présidente) a réuni un total de 102 participants du monde entier (France, Europe, USA, Canada, China, Australie, …), dont les 2 organisateurs, 25 conférenciers invités, 72 participants (étudiants en thèse, post-doctorants et chefs d'équipes), et 3 éditeurs de journaux scientifiques (EMBOJ, Nature Communication et Cell Reports).

Au cours de cette conférence les participants ont discuté des avancées les plus récentes concernant la fonction des ARN dans l'instabilité du génome, et en particulier 1) du rôle de la transcription comme menace pour l'intégrité du génome, 2) des conflits entre les machineries de réplication-transcription comme source d'instabilité du génome, 3) du rôle de la chromatine dans la réponse aux dommages à l'ADN, et dans le maintien de l'intégrité du génome, 4) des mécanismes de réparation des cassures double brins et notamment de l'implication des ARNs dans ces mécanismes de réparation.

Grâce à l'implication des participants, des orateurs invités (25 présentations) et des orateurs sélectionnés suite à leur candidature (19 présentations), la conférence a été un véritable succès et une occasion unique d'échanger autour de ce champ de recherche qui a récemment émergé.

The Jacques Monod Conference **"Genome instability: when RNA meets chromatin***"* was held in Roscoff, France on September 16-20, 2019. This conference, organized by Andrés Aguilera (Chair) and Gaelle Legube (vice-chair) brought the maximum possible of 102 participants, including the 2 organizers, 25 invited speakers, 72 applicants (PhD students, postdocs and a high number of PIs) from all over the world (France, rest of Europe, USA, Canada, China Japan, etc). In addition, 3 editors from different top journals (EMBO J, Nature Communications and Cell Reports) attended. 2 talks were supported by the EMBO YIP program. Altogether, this illustrates the attractiveness and high expectations of the conference.

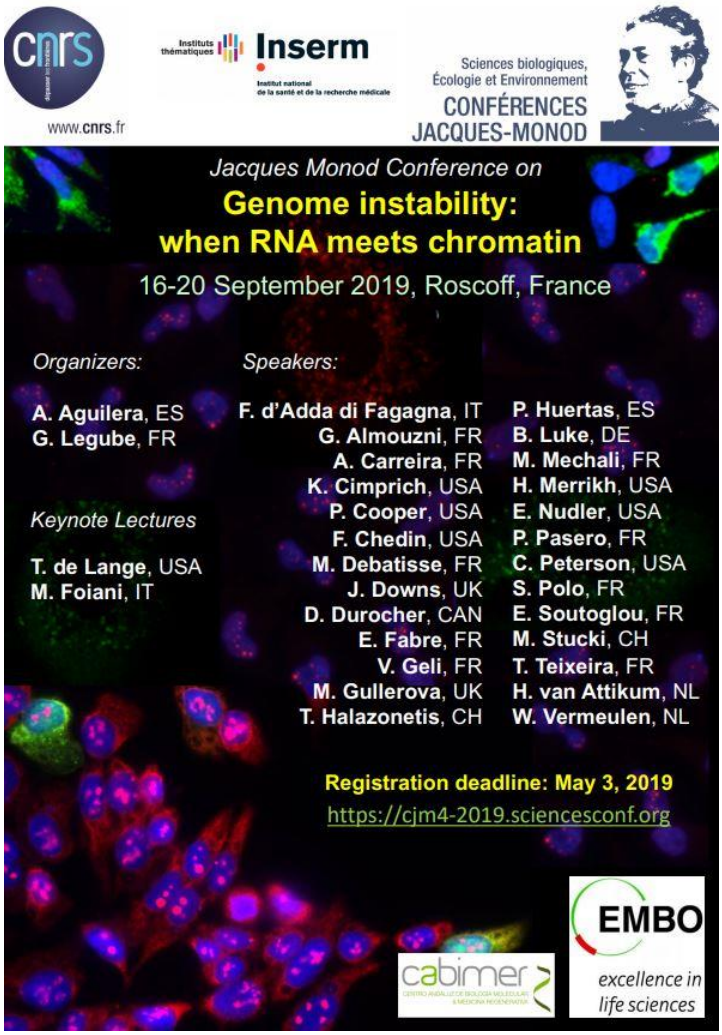
Aim of the Conference. The conference followed the one that took place in November 2016 in Roscoff on "**Transcription-Replication Crosstalk and Genome Instability**" organized by *Philippe Pasero* (Chair) and *Andrés Aguilera* (Vice-chair). Given the evolution of this field, the aim was to expand its focus to chromatin and the role that the RNA may play itself in DNA repair, thus integrating also the positive aspect that RNA may have on genome integrity. For this reason, the meeting was titled "**Genome instability: when RNA meets chromatin**". The new concept of the meeting tried to put together different fields including transcription, RNA metabolism and chromatin structure as major players in genome maintenance, putting definitively this topic in the center of the conceptual advances of Fundamental Biology and Biomedicine related to the causes of genome instability and disease. Related topics had been or will be partially covered in other meetings (Baeza 2014; Montpellier 2015; Roscoff 2016; Mainz 2018), but never as a whole integrating so many different processes. Thus, the final aim of this Jacques Monod Conference was to bring together scientists studying the DNA damage response/genome stability and RNA biology to discuss these different topics to decipher the interconnections between the genome integrity, RNA metabolism and chromatin to help establish a continuity of this meeting in Europe and America, in such an important field of research, in which there is a large community of researchers in Europe and, specifically, France. The conference came at the right time, as research in the last two decades placed replication and the DDR at the center of a complex interplay between DNA synthesis, transcription, RNA processing and epigenetics, with major consequences for genomic instability.

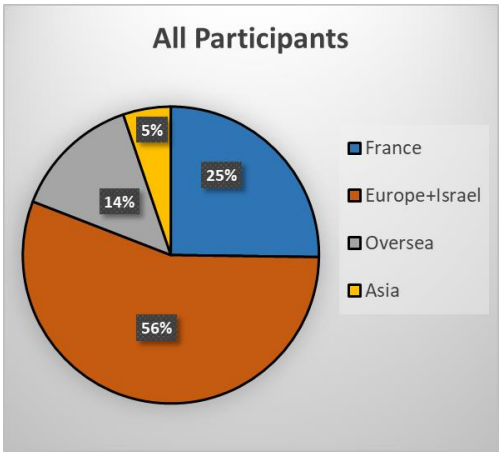
Conference overview. The conference started with the plenary lecture on Monday, September 16th after welcome drinks and a dinner at the Gulf Stream Hotel. Lectures were given in the auditorium of the Station Biologique CNRS. Part of the Thursday afternoon was devoted to an excursion to the "Ile de Batz" or free time. The conference ended on Friday, September 19th at 18:30 followed by a banquet. People departed early in the morning of September 20th

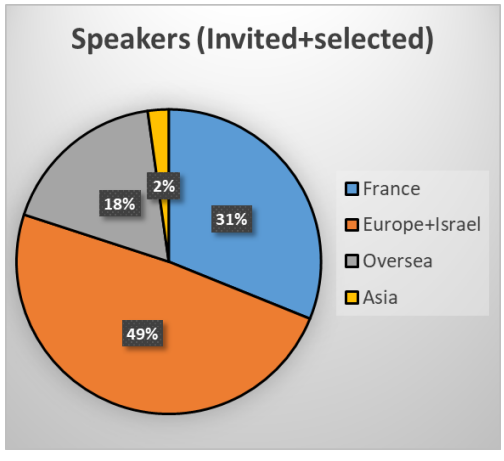
- 1 Plenary lecture (60 min)
- 6 sessions with a dedicated chair chosen among the invited speakers:
  - Session 1 – Transcription and RNA as threats
  - Session 2 – Replication conflicts
  - Session 3 – Chromatin and DNA damage response
  - Session 4 – Chromatin and genome integrity
  - Session 5 - Double strand break repair
  - Session 6 – RNA in DNA damage response
- 25 presentations by invited speakers in sessions (20 min + 5 min for questions)
- 19 short presentations selected from abstracts (12 min + 3 min for questions)
- Two poster sessions (2 hours each)

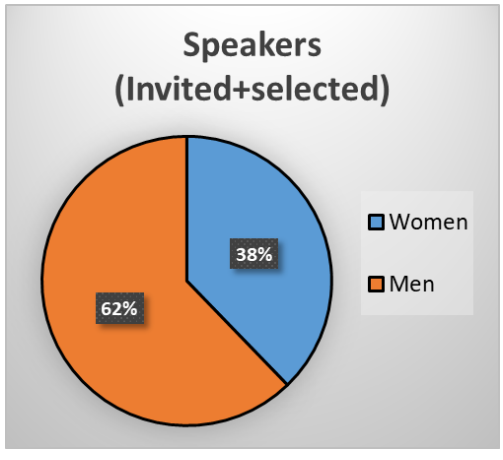
All aspects of management of the conference and the food were more than satisfactory, thanks to the expert care of Nathalie Babic and her colleagues. Overall, the conference was a big success, with a highly positive feedback of participants at both scientific and organization levels.

Conference statistics









Scientific program

Scientific context

Cell proliferation involves a number of processes that need to be tightly coordinated to ensure preservation of genome integrity and to promote faithful genome propagation. Coordination of DNA replication with DNA-damage surveillance mechanisms (termed checkpoints), repair and cell cycle progression ensure genome integrity during cell divisions. To maintain genome integrity, cells have evolved a network of biochemical and cellular reactions jointly known as the DNA damage response (DDR), that regulate different cellular processes, including replication, DNA repair, DNA-damage and cell cycle checkpoints and chromatin remodeling. Failures during replication and repair may lead to either mutation or to replication fork stalling and DNA breaks that would need to be repaired via recombination and that can lead to chromosomal rearrangements. Two types of elements play a key role in instability leading to rearrangements: those that act in *trans* to prevent instability – among them are replication, repair and S-phase checkpoint factors - and those that act in *cis* - chromosomal hotspots of instability such as Fragile sites and highly-transcribed DNA sequences. The accurate and complete duplication of the genome and maintenance of its primary sequence is crucial for normal cellular and organismal function. Changes in DNA sequence can ultimately lead to changes in the structure, function and/or expression of different proteins, altering numerous phenotypes. Genome instability is usually associated with pathological disorders, and in humans is often associated with premature aging, various cancer predispositions and hereditary diseases. Significant efforts over the past twenty years have focused on understanding how genome instability can arise.

Failures in DNA replication represent a major source of genomic instability. Importantly, high levels of spontaneous replication defects are detected in precancerous lesions as a consequence of oncogene-induced replication stress and play a central role in the cancer process. However, the origin and the consequences of this chronic replication stress remain poorly understood. In addition, a major source of DNA damage causing genome instability is a deficient DDR as can be manifested in defects in DNA repair as in the DNA damage checkpoint. The increasing link of mutations in DDR genes with genome instability in cancer cells and individuals with cancer- and premature aging-prone genetic diseases have placed these DNA metabolic processes in general as a major subject of research.

In the last decade a surprising interplay between the DDR and RNA biology has emerged. It has been shown that transcription and RNA processing can interfere with DNA replication, thus becoming a serious potential threat to genome stability. Reciprocally, DNA lesions able to interfere with replication and transcription globally impacts on different steps of RNA metabolism including transcription, RNA splicing and stability, as supported by the existence of specific DNA repair mechanisms coupled to transcription. In addition, recent observations suggest a potential important role of non-coding RNAs in DNA damage repair and signaling, which puts RNA as a key molecule in the whole network of DDR with both a potential positive and negative role in genome integrity. This role has been in the last years expanded by the increasing evidence showing that RNAs may have an important role in chromatin modifications that would affect DNA metabolic processes from replication to repair, apart of chromatin organization.

Altogether, research in the last two decades place replication and the DDR at the center of a complex interplay between DNA synthesis, transcription, RNA processing and epigenetics, with major consequences for genomic instability, which was discussed at the meeting.

Plenary lecture (Chair: Andrés Aguilera)

Marco Foaini gave an inspiring talk on how the topological structure of transcription was relevant for S phase. By genome-wide approaches and the use of different Topoisomerases tools they mapped genome-wide sites of topological constraints (negative supercoiling) generated during transcription that have an impact on replication progression, providing a novel model to explain how transcription and replication may modulate genome integrity.

Session 1 – Transcription and RNA as threats (Chair: Philippe Pasero)

In the first session we heard several talks on how transcription and RNA may modulate replication stress and chromatin structure, apart from how this RNA could form DNA-RNA hybrids along the whole genome. **Fred Chedin** provided new data on the genome wide-distribution of R loops showing how DRIP-seq and partial genome-wide analysis of R loops using bisulfite foot-printing providing evidence of coincidence of results and measurements of sizes and frequencies of R loops along the genome. He also presented evidence that R-loops absorb negative supercoils behind the RNAPII, acting as topological "sink". **Eugene Nudler** focused his talk on the cleavage factors GreA and GreB in bacteria and how ribosome controls transcription elongation rates by preventing backtracking. He discussed the model by which when the replisome hit a backtracked RNAP DSBs are generated. **Houra Merrikh** focused her talk on the role of mutagenesis in evolution and bacterial antibiotic resistance as well as the role of the Mfd transcription-coupled repair factor in mutagenesis. She further discussed a new role of TopoII in the homeostasis of R loops at transcription-replication conflicts in bacteria. **Qianwen Sun** presented a new work on how RNaseH1C and gyrases cooperate to control R loops in chloroplasts and new data relating 5meC at pericentromeric regions and how H2AW promotes chromatin condensation. **Karlene Cimprich** discussed the use of spike-ins in qDRIP analysis for DNA-RNA hybrid detection to focus the rest of the talk on the role of the mammalian HTLF factor of the Snf2 family of chromatin remodelers in replication stress and the possible role of PrimPol to understand how they can cause genome instability in different systems. **Natalia Gromak** gave an intriguing talk showing that RNH2 travels with RNAPII during transcription in mammalian cells. **Caroline Dean** revised her work on the nascent COOLAIR RNA providing new data on how it accumulates at G1/S phase and how after HU treatment the linked FLC silencing is lost.

Session 2 – Replication conflicts (Chair: Karlene Cimprich)

In the second session we moved a step further to focus on the mechanisms of replication and how they would bypass problems derived from collisions with different obstacles, including transcription. **Marcel Mechali** discussed the genetic and epigenetic features of mammalian DNA replication origins, providing data on nascent strand purification and sequencing. Data were originated from 19 seq experiments of 6 cell types from mice. He discussed the origin G-rich replication elements, heterochromatin origins and altered TAD border density of origins. **Thanos Halazonetis** provided data on variable boundaries of loci that display mitotic DNA synthesis (MiDAS), corresponding to latest replication origins. Interestingly they established a very clear correlation between MiDAS and transcriptional activity. **Jessica Downs** presented data on repressed transcription near DSBs and discussed new results on PBHF as promoter of SC cohesion and repair. She found cancer-associated mutants of STAG2 to be consistent for SC cohesion. Finally, she discussed data on H2A.Z dynamics at sites of DSBs. **Peter Stirling** gave a short talk on the role of PCNA on different aspects of genome instability and the role of transcription as a source of DSBs. **Aleix Bayona**, from Andrés Aguilera'slab, presented new data involving the SWI/SNF complex on the resolution of R-loop-dependent transcription-replication conflicts in human cells. **Michelle Debatisse** discussed her latest work on Common Fragile sites, their analysis on co-directional versus head-on transcription-collisions as a source of fragility, finding them irrelevant. **Philippe Pasero** reported new data using phosphorylated RPA to detect ssDNA accumulation. Comparative analysis of data from i-BLESS, OK-seq, DRIP-seq, RNA-seq

and others were presented to conclude that 3'-end and not 5'-end R loops those accumulating phosphorylated RPA. **Giovanni Tonon** showed data implying DIS3 in multiple myeloma and R loop homeostasis in connection with lower BRCA1 recruitment to IR induced breaks. **Nitika Taneja** presented an extensive work on the characterization of SMARCAD1 counteracting 53BP1 and its colocalization with PCNA to discuss a new role on stalled replication forks. **Batsheva Kerem** asked in her talk whether oncogenes could induce other form of replication stress, presenting data on the case of the RAS oncogene and its impact on TOP1 levels.

Session 3 – Chromatin and DNA damage response (Chair: Sophie Polo)

The third session discussed how the DNA damage response and repair mechanisms take place in chromatin and different nuclear compartment and chromosome structures. **Craig Peterson** presented new data on how promiscuous transcription causes genome instability, using high throughput sequencing methods to map DNA damage, R-loops and transcription. He then focused on the role of H3K56 acetylation and Rtt109/Asf1 that regulated such an acetylation. **Evi Soutouglou** presented her new data on how DSBs are repaired at centromeres. She showed that histone methyltransferase helps HR repair of DSBs at G1. **Manuel Stucki** presented novel data on how the TCOF1 Treacle protein via MRN recruitment to nucleoli affecting DSBs using I-PpoI expression to induce DSBs in the rDNA. **Dorthe H Larsen** showed that at enlarged nucleoli in cancer cells the TCOF1 phosphorylation by ATM is required to bring NBS1 to the nucleolus. She further reported the interested finding that DSB induced in rDNA does not activate a global checkpoint response. Finally, **Matthias Altmeyer** discussed new data on the response to replication stress in CHK1-induced replication catastrophe.

Session 4 – Chromatin and genome integrity (Chair: Craig Peterson)

In the fourth session we discussed the role of chromatin in genome dynamics preferably associated with transcription with emphasis at particular structures such as telomeres. **Enmanuelle Fabre** presented data on 3D-chromosome organization and chromatin mobility after DSBs generated by zeocin in yeast cells, showing that DSBs close to DSBs increases mobility but not at other sites. **Vincent Geli** presented data on the molecular mechanisms responsible for type I and type II survivors in telomerase-less mutants of yeast, presenting a novel model in which a nucleoporin Nup1 fused to *lexA* affected telomere dynamics. **Teresa Texeira** continue discussing data on replicative senescence after telomerase inactivation in yeast cells. She showed in addition that during senescence a mitochondrial dysfunction causes and increase in ROS. New data suggests that TERRA RNA is triggered in a Hog1-dependent manner after osmotic stress. **Jon Houseley** presented new data on the mechanism of CUP1 expansion in the yeast genome as a way to obtain cupper resistance. They showed that Mus81, Sae2 or Mre11 are required the extrachromosomal DNA being generated. **Sophie Polo** discussed her data on how HIRA promotes transcription restart after UV damage demonstrating that there is no correlation between histone H3.3 deposition and transcription restart after UVC. **Jeffery Daniel** presented his new work on CENPA and the different elements regulating its function. **Solene Hervé** showed that a rapid depletion of CENP-A in S-phase lead to rearrangements. Finally, **Sherif El-Khamisy** discussed new data on repeat expansions and the relation between DNA-RNA hybrids and autophay, with a focus on the role of USP11.

Session 5 - Double strand break repair (Chair: Fabrizio d'Adda di Fagagna)

Once discussed sufficiently transcription, replication and the nuclear context, in our try to get a general view on how DNA damage can be generated and can lead to instability, in the fifth session we discussed the recent advances in the mechanism of repair of the most important lesions, the double strand breaks (DSBs), which are believed to be the major responsible of the genome instability. **Monica Gullerova** presented new data on a Dicer pull-down showing that DICER associates with actively transcribed tRNAs. Dicer binds and processes alternatively

folded tRNAs to discuss bioinformatic analysis that predict target genes. **Haiko van Attikum** presented a new work on CHD7 showing that work at collisions between transcription and DNA repair mechanisms. There is a mutually exclusive localization of 53BO1 and CHD7-LIG4. Interestingly, CHD7 promotes XRCC4 NHEJ factor recruitment. **Aura Carreira** presented a new study showing that BRCA2 recruits a new DNA-RNA helicase DDX5 to remove potential DNA-RNA hybrids formed at DSBs. **Timothy Humphrey** show new data from *Schizosaccharomyces pombe* that IWS1 recruits SETD2 to RNAPII and that is required for DSB repair. Interestingly Iws1 recruitment is dependent on RNAPII Ser2P. **Boris Pfander** presented his new work where they established the AsiSI-based system in yeast and identify 39 break sites in the yeast genome. He further presented their lack of evidence for histone assembly on single stranded DNA using genome wide approaches. **Daniel Durocher** presented new data on the BTAF1 homolog to Mot1 and PARP1-BRCA synthetic lethality. Interestingly they show that loss of RNH2 is lethal in BRCA2-deficient cells. **Gaelle Legube** presented new data on the recruitment of SETX to DSBs generated in the DIvA system previously developed by her. Interestingly she also presented a new study on ribosomal DSB repair showing that HUSH-H3K9me3 methyltransferase mediates DSB-induced transcription. **Oliver Sordet** studied quiescent cells and G1 cells treated with CPT to demonstrate that Top1cc increases R loops over gene bodies and that XPF, XPG and FEN1 contributes to the formation of DSBs after an R loop. Finally, **Domenico Libri** presented a new story on the Nab3-Sen1-Nrd1 yeast complex involved in transcription termination

Session 6 – RNA in DNA damage response (Chair: Daniel Durocher**)**

In the final session we discussed the recent results generated on the new role that the RNA itself may have in DSB repair. **Pablo Huertas** presented his new work showing a role of ADAR2 on the removal of DNA-RNA hybrids at DSBs to allow repair. **Fabrizio d'Adda di Fagagna** presented data showing the synthesis of ncRNAs at the site of DNA damage and the potential role of DSB as "promoters". **Wim Vermeulen** discussed his new work on the role of chromatin remodelers such as SWI/SNF on GTF2 expression and TFIIH action, which would promote DDB2 release from damage to allow NER. **Brian Luke** presented his new work on TERRA R loops in yeast cells and how they delayed or promoted senescence depending on their levels. Finally, **Kazuko Nishikura** presented her work on the role pf ADR1 on DNA-RNA hybrid homeostasis.

The conference was a scientific success attending all comments and feedback of participants during and after the meeting.

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